

## ORIGINAL ARTICLE

# Dark ground microscopy and treponemal serological tests in the diagnosis of early syphilis

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**Objectives:** To evaluate the use of dark ground microscopy (DGM) and treponemal serological tests in the diagnosis of primary (PS) and secondary (SS) syphilis.

**Methods:** A retrospective case note review of patients with early syphilis who attended our department between January 2001 and December 2002. Data were collected on demographics, results of treponemal serology and DGM.

**Results:** 50 individuals had PS and 36 individuals had SS. DGM was performed in 31/50 (62%) of PS cases and this was positive in 97%. In 17 (34%) cases of PS, treponemal EIA was negative initially. DGM was performed on 13 of these, all of which were positive. Therefore, EIA had a sensitivity of 57% when compared to DGM. In 27 patients where EIA-IgM was performed, this was positive in 22 (81%), of which 12 were EIA negative on initial screening. All SS cases had positive EIA. DGM was performed in 19/36 (52%) of SS cases and was positive in 16/19—that is, a sensitivity of 84% when compared to EIA. The major reason why DGM was not performed in the cases of PS was that herpes was the presumed diagnosis and in SS the rash was attributed to other causes.

**Conclusions:** DGM is a rapid and sensitive test while EIA takes time for results and is less sensitive in PS. EIA-IgM is a useful adjunct in PS. DGM allows immediate diagnosis, treatment, and partner notification preventing further transmission. Genitourinary medicine clinics should have trained staff to perform DGM on all anogenital ulcers and suspected syphilitic lesions.

The recent increase in the incidence of infectious syphilis, especially in homosexual men, is of public concern. The outbreaks of syphilis in Bristol, Brighton, Manchester, Edinburgh, Cambridgeshire, and London<sup>1–6</sup> emphasise the importance of its early diagnosis in order to prevent the spread of this infection. The diagnosis of primary and secondary syphilis can be made using direct detection methods such as dark ground microscopy (DGM), direct fluorescent antibody (DFA-Tp) staining, or polymerase chain reaction (PCR) and serological tests. In patients with primary syphilis (PS), serological tests for syphilis such as treponemal enzyme immunoassay (EIA) and reaginic tests such as rapid plasma reagin (RPR) are not sensitive. In 30% of these patients the RPR will be non-reactive<sup>7</sup> and EIA tests have been shown to have sensitivities ranging from 48.5–76.9% when testing highly selected sera with a negative TPHA in patients with PS.<sup>8</sup>

DGM has the advantage in detecting infection early and on site. The need for such a test is important because it allows treatment to be initiated immediately, enables early partner notification, and therefore prevents spread of infection. However DGM requires a dark field microscope and trained personnel to perform it. Indeed, before the current outbreak of syphilis, many genitourinary medicine physicians had seen very little syphilis and therefore are not able to perform this technique, as was the case in the Manchester studies.<sup>4–9</sup>

An alternative direct detection method is the DFA-Tp staining method. This does not require live organisms, can be used for oral lesions and is as sensitive and specific as DGM.<sup>10</sup> However this technique is limited as the monoclonal reagents required are not available commercially, a special microscope is needed, and it is more time consuming than DGM. PCR is a highly sensitive and specific technique for the detection of PS and to a lesser extent SS,<sup>9–11</sup> but requires specimens to be sent to the laboratory and may take some time for results.

The aim of this study is to evaluate the use of DGM and treponemal serological tests in the diagnosis of primary and secondary syphilis, and if DGM was not performed, to establish the reasons why.

## METHODS

We performed a retrospective case note review of patients diagnosed with primary (PS) or secondary syphilis (SS), who attended the Ambrose King Centre between January 2001 and December 2002; 86 patients were identified using KC60 coding. The diagnosis of PS was based on the presence of anogenital or extragenital ulcer and positive DGM from these sites and/or serological tests for syphilis, whereas the diagnosis of SS was based on clinical signs and positive syphilis serology and/or DGM. Data were collected on demographics (sex, sexuality, age, HIV status), stage of syphilis, syphilis serology, and dark ground microscopy (site, result, and reason if not performed).

## Dark ground microscopy

Specimens were collected for DGM from suspected syphilitic lesions. The sites of the primary lesions were located on the penis, anus and outer lip. When specimens were taken from the mouth, care was taken to remove any saliva which might have contained oral non-syphilitic treponemes. An experienced person in DGM reviewed the oral specimen to differentiate between pathogenic and commensal treponemes. Secondary syphilitic lesions were located on the penis, scrotum, vulva, and extragenital skin. The basic principles of DGM have not changed over the years<sup>12–13</sup> and

**Abbreviations:** DFA-Tp, direct fluorescent antibody; DGM, dark ground microscopy; EIA, enzyme immunoassay; PCR, polymerase chain reaction; PS, primary syphilis; RPR, rapid plasma reagin; SS, secondary syphilis

the technique used in this study is described in table 1. Although lymph node aspirate can be used for DGM<sup>12</sup> it was not performed in this study. At least three specimens were collected for DGM and all slides were examined within 5–10 minutes of collection using a dark field microscope. If negative at first, DGM was repeated daily for at least three consecutive days if this was feasible.

### Serological tests

All patients had a treponemal EIA (ICEsyphilis, Abbott-Murex) as a screening test and if positive RPR (RPR reitit, Biokit) and TPPA (Seroda-TPPA, FujiRebio) were performed. Samples were sent to the Health Protection Agency (HPA) Reference Laboratory (Bristol) for EIA-IgM if there were discrepancies between the tests or if it was specifically requested by the doctor when primary syphilis was suspected.

## RESULTS

### Patient characteristics

The patient characteristics of the study population are shown in table 2. The majority of the cohort were homosexual men (75%) and 30% of these were HIV positive.

### Primary syphilis

We identified 50 cases of PS, nine of whom were known to be co-infected with HIV. DGM was performed in 31/50 (62%) of the cases of PS and this was positive in 30/31 (97%). Syphilitic lesions were located on the genitalia in 23 of the DGM positive patients, the anus in five patients, and on the external lip in two patients. In 24 cases, DGM was found to be positive on the first visit—that is, 48% of all cases of PS. DGM was not performed in 19 individuals (six of these were co-infected with HIV). The reasons for this were: in six cases (two HIV positive) the ulcers were painful and anogenital herpes simplex virus infection was presumed to be the diagnosis; seven cases (two HIV positive) had atypical ulcers and syphilis was not suspected; in four cases no trained personnel were available; in one case the DG microscope was not working and in the final case, the lesion was located on the inner lip and therefore DGM might have given unreliable results because of commensal treponemes. In 27 cases of PS, blood samples were sent to the HPA Reference Laboratory. Of these, treponemal EIA-IgM was positive in 21 cases,

**Table 2** Demographic details of cohort

Variables	No (%)
Total	86
Sex	
Female	2
Male	84
Sexual orientation	
Heterosexual	22 (25)
Homosexual	64 (75)
HIV status	
Positive	19 (22)
Negative	24 (49)
Not known*	25 (29)
Ethnicity	
White	70
Afro-Caribbean	5
African	2
Asian	1
Black other	2
Other	5
Unknown	1

\*No HIV test performed within 6 months of syphilis diagnosis.

equivocal in four cases, and negative in two cases. Of the EIA-IgM negative cases, IgG was positive in one case and in the other case all serology was negative; however, the patient was a contact of a documented early syphilis case. DGM was performed (and found to be positive) in three equivocal EIA-IgM cases and one of the negative EIA-IgM cases.

In 17 (34%) cases of PS, treponemal EIA was negative initially. DGM was performed on 13 of these, all of which were positive. Therefore, EIA had a sensitivity of 57% when compared to DGM. Samples were sent to the HPA reference laboratory in 14/17 of the EIA negative cases, and EIA-IgM was positive in 12/14 of these. The median length of time between the patients' initial presentation and a positive serological test was 10 days (4–60 days). However, if the initial EIA was negative this increased to a median time of 32 days (range 5–60 days).

### Secondary syphilis

We identified 36 cases of SS. Ten of these were co-infected with HIV. DGM was performed in 19 (52%) of the 36 cases of SS. This was positive in 16/19 (84%). The syphilitic lesions were located on the genitalia in seven cases (four were persisting primary chancres) and in the other nine cases the lesions were extragenital, located on the skin.

DGM was positive on the first visit in 15 cases—that is, 42% of all cases of SS. DGM was not performed in 17 individuals. The reasons for this were: in nine cases the rash was attributed to other causes—for example, drug rash; in two HIV positive cases the individuals were initially asymptomatic, however routine syphilis serological testing was found to be positive (they both later developed syphilitic lesions); in five instances no trained staff were available; and in the last case, the reason was not documented.

All cases of SS had positive initial treponemal serology. DGM therefore, had a sensitivity of 84% when compared to EIA. The median time interval from initial visit to positive serology was 6 days (0–59 days).

## DISCUSSION

The diagnosis of early syphilis is based upon a high index of clinical suspicion, serological tests and/or identification of *Treponema pallidum* in clinical specimens. A diagnosis made clinically can be problematic: genital ulceration has several differential diagnoses and signs and symptoms of SS are diverse and may not be recognised. In an STD clinic in the United States, in which the prevalence of syphilis was high,

**Table 1** Dark ground microscopy

#### Obtaining the specimen

- Cleanse lesion with saline using gauze swab
- Dry and abrade with dry gauze swab; or abrade with scalpel for intact papule or healing ulcer if necessary
- Squeeze clear serum—do not dilute with saline
- Collect oozing serum with edge of coverslip
- Put coverslip onto slide
- Press between filter paper evenly

#### Dark field microscope

- Use a dark field condenser
- Place 1 drop of oil on bottom of slide where the specimen is
- Put slide on the stage ensuring oil contact between condenser and slide
- Objective ×10
  - Focus
  - Close field diaphragm and centre the circular light beam with centring screws
  - Open field diaphragm
- Objective ×40
  - Search
- Objective ×100
  - Place oil on top of the coverslip before using objective.
  - Adjust the iris diaphragm in the objective for "darkness" of field
  - Detail identification of spirochaetes looking for characteristic morphology and motility

the positive predictive value of a clinical diagnosis of PS, based on lesion morphology alone, was only 78%.<sup>14</sup>

If serological tests are to be used alone several problems may be encountered. Differentiation of past infection from a current infection may be difficult. A negative RPR in early syphilis or a positive EIA or TPHA owing to past or inadequately treated syphilis may mislead the clinician. The RPR test can also yield false negative results in the presence of high titres of antibody (prozone phenomena). This might be seen in early syphilis or when there is concomitant HIV infection.

Treponemal EIA is recommended as a screening test in the United Kingdom<sup>15</sup> and it is a sensitive test when testing sera from patients with various stages of syphilis.<sup>16</sup> However it is a significantly less sensitive test in PS.<sup>8</sup> We demonstrated similar results; EIA had a sensitivity of 57% when compared to DGM in PS, whereas EIA was positive in all cases of SS. Another problem with EIA is that it may yield a reduced or a delayed response when there is concomitant HIV infection.<sup>17</sup> Studies have shown that EIA-IgM becomes positive earlier in PS compared to treponemal EIA,<sup>10</sup> but EIA-IgM needs to be sent to a reference laboratory and this can cause delays in diagnoses. However, IgM is a useful adjunct for detection of primary syphilis and should be requested in patients with suspected primary syphilis.

DGM is a sensitive test for the demonstration of *T pallidum* and is specific.<sup>18–19</sup> Although fluorescent antibody techniques for the identification of *T pallidum* have sensitivities and specificities that are comparable to DGM,<sup>18–19</sup> they tend to be more time consuming than DGM and cannot be performed on site.

In our study, of the 50 patients in whom DGM was performed, 46 (92%) had a positive DGM. Thirty nine cases had a positive DGM on the initial visit—that is, 45% of the total cohort, thus treatment could be prescribed immediately. In 13 cases, DGM was positive but the initial EIA was negative, and in four of these cases the IgM was equivocal or negative. If DGM had not been performed, delay of treatment or more seriously, missed diagnoses could have occurred. A worrying finding was the time delay between initial presentation and positive serology. This was a median of 32 days if the initial serology was negative. Fortunately, DGM was performed in the majority of these individuals and therefore the delay in treatment was avoided.

DGM was performed in 58% of all cases in our study. The major reason why it was not performed in the cases of primary syphilis was that either the ulcers were painful, and therefore HSV was the presumed diagnosis, or that the lesions were atypical of syphilitic ulcers. Many of the cases of PS in the north Manchester study<sup>4</sup> presented with painful multiple sores more typical of HSV. These, and other atypical presentations, especially in HIV infected patients,<sup>20</sup> may reflect a changing presentation of PS and emphasise the point that all genital ulcers should have DGM performed even when herpes simplex virus is suspected.

The most common reason DGM was not performed in the cases of SS was because the rash was attributed to other causes, such as drug eruption, particularly in HIV infected patients on antiretroviral medication. Syphilitic skin rashes may imitate any generalised eruption (except vesicular or bullous)<sup>12</sup> and therefore SS should be considered in all patients with a generalised rash attending especially in the GU/HIV setting.

The use of DGM may be limited. Microscopes equipped with dark field are unavailable in non-genitourinary medicine settings and therefore DGM is restricted to the genitourinary medicine clinic. Another limitation is the availability of trained staff in the use of DGM, as was the case in the north Manchester study.<sup>4–9</sup> This was also a problem in

## Key messages

- Dark ground microscopy is an inexpensive, rapid, sensitive, and specific test, which allows immediate diagnosis and treatment of syphilis
- All anogenital ulcers including suspected genital herpes should have DGM
- Staff in genitourinary clinics should be trained in the technique of DGM

our study, as there were nine instances when DGM was not performed for this reason and this had led us to establish training for our staff in DGM techniques.

In conclusion, DGM is an inexpensive, rapid, sensitive, and specific test, which allows immediate diagnosis and treatment of syphilis, preventing further transmission and enabling early partner notification. DGM should be performed on all anogenital ulcers, external oral ulcers, and other suspected syphilitic lesions. The training of all staff, doctors, and nurses, in DGM is an absolute priority in all genitourinary medicine clinics and a specific DG microscope should be set up for immediate use, especially in the current outbreak of syphilis. Treponemal EIA is highly sensitive in SS but less so in PS, while EIA-IgM is a useful adjunct for the diagnosis of primary syphilis.

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## CONTRIBUTORS

HLW participated in the protocol development, collected the data, and was the principal author; SA assisted with data collection and helped with the literature search; BTG initiated the study, developed the protocol, trained the staff in DGM, and critically revised the paper.

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